85. (New) The method of claim 1 wherein the agent is an ubiquitin ligase inhibitor.

86. (New) The method of claim 1 wherein the agent alters endosomal processing.

Remarks

Reconsideration and withdrawal of the objections to the specification and rejections of the claims, in view of the remarks and amendments herein, is respectfully requested. Claims 1, 6, and 12 are amended, and claims 85-86 are added; as a result, claims 1-86 are now pending. The amendments are intended to further prosecution and are not intended to concede to the correctness of the Examiner's position or to prejudice the prosecution of the claims prior to amendment, which claims are present in a continuation application of the present application.

Amended claim 1 is supported, e.g., by originally-filed claim 1 and by page 4, lines 19-21 and 29-31, page 20, lines 23-33 and page 21, lines 3-6 of the specification.

Amended claim 6 is supported by originally-filed claim 6.

Amended claim 12 is supported by originally-filed claim 12 and by page 21, lines 24-33 of the specification.

New claim 85 is supported by page 8, line 9 of the specification.

New claim 86 is supported by originally-filed claim 1 and by page 4, lines 29-31, page 4, line 31-page 5, line 3, page 5, lines 11-18, and page 8, lines 7-9 of the specification.

The amendments to the "Brief Description of the Figures" address the objection to the specification noted at page 5 of the Office Action.

The substitute drawings enclosed herewith moot the objections to the drawings noted on Form PTO 948.

The 35 U.S.C. § 112 Rejections

The Examiner rejected claims 7-8 and 12 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the

subject matter which Applicant regards as the invention. This rejection, as it may be maintained with respect to the pending claims, is respectfully traversed.

In particular, the Examiner asserts that 1) the phrase "endosomal protease inhibitor" in claim 7 is unclear because it is not a term of art and the specification is silent regarding the properties of an endosomal protease inhibitor, and 2) the phrase "a marker gene or a selectable gene" in claim 12 is indefinite. The amendments to claim 12 render the § 112(2) rejection of that claim moot.

With respect to the phrase "endosomal protease inhibitor", it is Applicant's position that the phrase was conventionally used and understood by the art at the time of Applicant's filing. For instance, the abstract of Fiani et al. (Archives Biochem. Biophys., 307:225 (1993), a copy is enclosed herewith) discloses that "[i]nhibition of endosomal proteases such as cathepsin D and B" lead to the accumulation of ricin A toxin inside of macrophages and the abstract of Adorini et al. (J. Immunology, 151:3576 (1993), a copy is enclosed herewith) discusses "the endosomal protease inhibitor leupeptin" in preventing presentation of an epitope. Thus, the phrase "endosomal protease inhibitor" in claim 7 is clear.

Hence, Applicant respectfully requests withdrawal of the rejections under 35 U.S.C. § 112, second paragraph.

The 35 U.S.C. § 102 Rejections

The Examiner rejected claims 1, 4-5 and 10-12 under 35 U.S.C. § 102(b) as being anticipated by any one of Alexander et al. (U.S. Patent No. 5,604,090), Russell et al. (Proc. Natl. Acad. Sci. USA, 92:5719 (1995)), Halbert et al. (J. Virol., 71:5932 (1997)), Qing et al. (Proc. Natl. Acad. Sci. USA, 94:10879 (1997)), or Kessler et al. (Circulation, 92:I-296 (1995)). These rejections, insofar as they may apply to the pending claims, are respectfully traversed.

Alexander et al. generally disclose a method for increasing the efficiency of transduction of cells by recombinant adeno-associated viral (rAAV) vectors, which employs agents that alter DNA metabolism (abstract), and a method of screening for a transduction increasing agent which employs rAAV and an agent that alters DNA

metabolism (column 3, lines 1-14). Alexander et al. indicate that agents that alter DNA metabolism "as defined herein include those whose principal mechanism or modes of action are directed to DNA metabolism, not those whose effect is remote" (column 4, lines 42-45). In particular, the agents are disclosed as ones which affect DNA synthesis and/or repair, impact maintenance of chromosomal integrity, and/or cause damage to cellular DNA, e.g., radioactive thymidine and gamma irradiation, which cause DNA scission; UV irradiation, which causes DNA dimerization; cis-platinum, which causes DNA alkylation; etoposide, a topoisomerase inhibitor, which disrupts chromosomal integrity; and hydroxyurea, a ribonucleotide reductase inhibitor, and aphidicolin, a DNA polymerase inhibitor, which affect DNA synthesis (column 4, lines 61-67 and column 5, lines 2-10). For instance, cells contacted with rAAV and radioactive thymidine, gamma irradiation, UV irradiation, cis-platinum, etoposide, hydroxyurea, or aphidicolin showed increased AAV transduction efficiencies (Examples 1, 4-6, 9, and 11-12), while cells contacted with rAAV and nocodaxole, a cytotoxic agent that inhibits mitotic spindle formation, and methotrexate, a cytotoxic agent that is folic acid antagonist, showed no or little effect on AAV transduction efficiency (Example 2).

Russell et al. disclose that aphidicolin or hydroxyurea (both inhibitors of DNA synthesis) and some topoisomerase inhibitors, i.e., etoposide and camptothecin, but not others, i.e., novobiocin and amsacrine, increased rAAV transduction of human fibroblasts (page 5720). It is also disclosed that inhibitors of RNA or protein synthesis, i.e., actinomycin D and cycloheximide, did not increase rAAV transduction (page 5720). Table 1 in Russell et al. shows that combinations of tritiated thymidine, hydroxyurea, etoposide and camptothecin "did not increase transduction significantly more than the most effective agent" (page 5721). The results, according to the authors, "suggest a common mechanism for the effect, as does the lack of additive transduction effects provided by treatment combinations" (page 5722).

Russell et al. suggest that "[*e*]*x vivo* gene transfer protocols [with AAV vectors] could include treatment with DNA synthesis or topoisomerase inhibitors" (page 5723). However, the infusion of etoposide to rabbits concurrent with intrabronchial

administration of rAAV did not improve rAAV transduction rates in the airway of those rabbits (Halbert et al.).

With regard to the abrogation of the inhibitory effect of wild-type AAV on rAAV by etoposide *in vitro*, Halbert et al. disclose that etoposide "presumably does not affect viral entry but acts at later steps in transduction" and "may increase factors involved in DNA synthesis and repair" (page 5940).

Figure 5 in Qing et al. shows that hydroxyurea and genistein (a protein tyrosine kinase inhibitor) increased rAAV transduction of HeLa cells by 60% while sodium ortho-vanadate (a protein phosphatase inhibitor), increased rAAV transduction by only 3% (page 10882).

Kessler et al. relate that sodium butyrate, "a potent modifier of gene expression", increased the transduction efficiency of cardiomyocytes by rAAV by 50%. Sodium butyrate, a histone deacetylase inhibitor (Kruh, Mol. Cell. Biochem., 42:65 (1982), a copy is enclosed herewith) was known to interact with the cytoskeleton, induce protein synthesis, support viral DNA replication, and activate integrated viral DNA.

Nevertheless, none of the cited art discloses a method to identify agents which alter AAV transduction, wherein the agent alters transduction after viral binding to receptors and before synthesis to an expressible form of the AAV genome. Accordingly, withdrawal of the rejections of the claims under 35 U.S.C. § 102(b) is respectfully requested.

The 35 U.S.C. § 103 Rejection

The Examiner rejected claims 1, 3-5 and 10-11 under 35 U.S.C. § 103(a) as being unpatentable over Alexander et al. in view of Snyder et al. (WO 98/24479). As this rejection may apply to the pending claims, it is respectfully traversed.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, the prior art reference (or references when combined) must teach or suggest all the claim limitations. Second, there must be some suggestion or motivation, either in the cited references themselves or in the knowledge generally available to an art worker, to

modify the references or combine reference teachings so as to arrive at the claimed invention. Third, the art must provide a reasonable expectation of success. M.P.E.P. § 2143. The teaching or suggestion to arrive at the claimed invention and the reasonable expectation of success must both be found in the prior art, not in Applicant's disclosure (M.P.E.P. § 2143, citing with favor *In re Vaeck*, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991)).

Snyder et al. disclose methods of expressing polynucleotides in the cells of the liver using rAAV (abstract). It is disclosed that the rAAV is modified to reduce the possibility of rescue with a wild-type herpesvirus or adenovirus, e.g., by deleting or otherwise inactivating viral genes, and that the rAAV may be used alone or in conjunction with agents that enhance transduction, i.e., gamma irradiation, UV irradiation, tritiated nucleotides, cis-platinum, etoposide, hydroxyurea, camptothecin, aphidicolin and adenovirus (pages 11 and 23).

Thus, Snyder et al. do not supply what is missing in Alexander et al., i.e., neither reference teaches or suggests detecting or determining an agent that alters virus transduction, wherein the agent alters transduction after viral binding to receptors and before synthesis to an expressible form of the AAV genome.

Moreover, assuming, for the sake of argument, the art worker would be motivated to identify agents that alter rAAV transduction, the cited references do not provide a reasonable expectation that agents other than those disclosed in the cited art would be effective to alter AAV transduction of mammalian cells.

Accordingly, Applicant respectfully requests withdrawal of the 35 U.S.C. § 103(a) rejection.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-373-6959) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

JOHN F. ENGELHARDT ET AL.,

By their Representatives,

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Date November 26, 2002 By _____
Janet E. Embretson
Reg. No. 39,665

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on this 26th day of November, 2002.

Candis B. Buending
Name

_____
Signature

CLEAN VERSION OF AMENDED SPECIFICATON PARAGRAPHS

Please replace the paragraph beginning at page 11, line 9 with the following paragraph:

Figures 3A and 3B. Polarized airway epithelia were treated with UV (25 j/m^2) prior to infection with AV.GFP3ori virus (MOI = 10,000 particles/cell) from the apical (Figure 3A) or basolateral side (Figure 3B) of primary cultures. The abundance of GFP transgene expressing cells was quantitated by indirect fluorescent microscopy at 4, 8, 22, 30, and 40 days. The results represent the mean (+/- SEM) of 4 independent experiments for each condition.

C1

Please replace the paragraph beginning at page 12, line 13 with the following paragraph:

Figures 7A and 7B. Proteasome inhibitors differentially augment rAAV transduction from the apical or basolateral surfaces of airway epithelia. The efficiency and time course of rAAV transduction were evaluated in polarized airway epithelial cultures following infection with rAV.GFPori3 (MOI=10,000 particles/cell) in the presence or absence of 40 μM LLnL. Transgene expression was monitored by indirect fluorescence microscopy at the indicted time points by quantifying the mean number of GFP positive cells per 10 x field (mean +/- SEM of 3 independent samples for each time point). The effect of LLnL treatment was compared between matched sets of tissue samples at each time point following infection from the apical (Figure 7A) or basolateral (Figure 7B) surfaces. The photomicrographs on the right side of each figure illustrate representative 20 x fields for the 3 and 22 day post-infection time points.

C2

Please replace the paragraph beginning at page 13, line 32 with the following paragraph:

C3

Figures 11A and 11B. Optimization of LLnL-enhanced transduction in polarized bronchial epithelia. Differentiated transwell cultures were infected with AV.GFP3ori (10,000 particles/cell) from either the basolateral (Figure 11A) or the apical (Figure 11B)

surface with the indicated treatments involving LLnL and/or EGTA. All infections were carried out for 24 hours and GFP transgene expression was monitored by indirect fluorescent microscopy at the indicated times. Data represent the mean (+/- SEM, N=6) for each experimental condition. Experiments were performed in triplicate on transwells derived from samples obtained from two different patients. The following conditions were evaluated for basolateral infection in Figure 11A: 1) single infection with AV.GFP3ori alone (black line), 2) single infection with AV.GFP3ori in the presence of 40 μM LLnL (solid purple line), 3) single infection with AV. GFP3ori in the presence of 40 μM LLnL followed by repeated 5 hour exposure to 40 μM LLnL in the basal compartment culture medium every 3rd day thereafter (solid red line), 4) single infection with AV.GFP3ori in the presence of 40 μM LLnL followed by the continued exposure to 40 μM LLnL in the basal medium after rAAV was removed (solid green line), and 5) repeated infection with AV.GFP3ori on day 1 and 15 in the presence of 40 μM LLnL for 24 hours at the time of infection (dashed blue line). The following conditions were evaluated for apical infection in Figure 11B: 1) single infection with AV.GFP3ori alone (solid black line); 2) single infection with AV.GFP3ori following pretreatment with 3 mM hypotonic EGTA prior to the viral infection (solid purple line); 3) single infection with AV.GFP3ori in the presence of 40 μM LLnL (solid green line); and 4) single infection with AV.GFP3ori in the presence of 40 μM LLnL following pretreatment with 3 mM hypotonic EGTA prior to the viral infection (solid red line).

C3 cont'd

Please replace the paragraph beginning at page 14, line 25 with the following paragraph:

C4

Figures 12A and 12B. Binding and uptake of S^{35}-labeled AV.GFP3ori in fully differentiated human bronchial epithelia. The ability of polarized bronchial epithelia to bind and internalize virus from the apical or the basolateral surfaces was quantified using S^{35}-labeled rAAV. The binding assay was performed after incubation with virus at 4°C for 1 hour, followed by repeated washing in PBS. The combined bound and internalized virus was quantified following incubation with virus at 4°C for 1 hour, and subsequent incubation at 37°C for 2 hours and 24 hours. Non-specific background binding of

radiolabeled virus was determined in parallel studies on collagen coated empty chambers not seeded with bronchial cells. Background counts (averaging 15.67 +/- 5.17 cpm/well) were subtracted from experimental sample counts prior to analysis. Data in the right side of each figure is presented as the net cpms of bound/internalized virus (raw counts minus background counts of empty transwells). The results represent the mean (+/- SEM) of 6 independent transwells for each condition. Experiments were performed in triplicate from two independent tissue samples. The significance of the differences between each pair of samples (with or without LLnL) was evaluated using the Student's t-test and p-values are provided in brackets above the data for each condition. To correlate uptake of radioactive virus with the functional expression the rAAV encoded transgene, GFP expression from the same set of samples was quantified at 24 hour post-infection by indirect fluorescent microscopy. The results (Mean +/- SEM, N=6) are presented as a bar graph on the right side.

Please replace the paragraph beginning at page 16, line 14 with the following paragraph:

Figures 15A-C. Examination of rAAV endocytosis by Southern blot analysis of viral DNA. Hirt DNA from AV.GFP3ori infected or mock infected (Lanes 1 and 7 in both Figures 15A and 15B) human bronchial epithelia were extracted for a direct examination of viral genomes by Southern blotting against a P^{32}-labeled EGFP probe. Figure 15A depicts viral binding studies in the presence and absence of LLnL with or without EGTA treatment prior to apical or basolateral infection for 1 hour at 4°C. Cell surface-bound virus was completely removed by trypsin (Figure 15A, lanes 2 through 6). To determine the amount of the surface-bound rAVV, cells were infected with AV.GFP3ori for 1 hour at 4°C and were not treated with trypsin prior to Hirt DNA extraction. Panel A: lane 8: apical AAV infection; lane 9: apical AAV infection in the presence of LLnL; lane 10: cells were pre-treated with hypotonic EGTA prior to apical infection in the presence of LLnL; lane 11: basolateral infection; lane 12: basolateral infection in the presence of LLnL. Figure 15B depicts the results of studies evaluating rAAV internalization from either the apical or the basolateral surface in the presence or

absence of LLnL, and the internalization from the apical surface after combined treatment with hypotonic EGTA and LLnL. To detect the net amount of the internalized viral genome, all samples in Figure 15B were treated with trypsin just before Hirt DNA was harvested. The extent of the internalized virus at 4 hours (Figure 15B, lanes 2 through 6) and 24 hours (Figure 15B, lanes 8 through 12) incubation at 37°C after infection is represented by the intensity of the 1.6 kb single stranded viral genome band. Figure 15B; lane 2; apical AAV infection for 4 hours; lane 3: apical AAV infection in the presence of LLnL for 4 hours; lane 4: cells were pre-treated with hypotonic EGTA prior to apical infection in the presence of LLnL for 4 hours; lane 5: basal infection for 4 hours; lane 6: basolateral infection in the presence of LLnL for 4 hours; lane 8: apical infection for 24 hours; lane 9: apical infection in the presence of LLnL for 24 hours; lane 10: cells were pre-treated with hypotonic EGTA prior to apical infection in the presence of LLnL for 24 hours; lane 11: basolateral infection for 24 hours; lane 12: basolateral infection in the presence of LLnL for 24 hours. Figure 15C compares the effect of LLnL/EGTA on rAAV genomes at 2, 10, 30 days following a 24 hour infection from the apical (lanes 1, 2, 5, 6, 10, 11 and 12) and basolateral (lanes 3, 4, 7, 8, 9, 13 and 14) membranes. Treatment conditions are noted above each lane; transwells were not treated with trypsin prior to harvesting Hirt DNA. An additional control included co-infection with Ad.d1802 (MOI=500 part/cell) to demonstrate replication form monomers (lane 9, 4.7 kb). It should be noted that different exposure times were used for the three different panels in Figure 15C (lanes 1-4, 3 hours; lanes 5-8, 15 hours; lanes 10-14; 12 hours). Matched DNA samples from uninfected cultures did not demonstrate detectable hybridization (data not shown).

C5 cont'd

Please replace the paragraph beginning at page 17, line 21 with the following paragraph:

C6

Figures 16A-D. Modification of the viral ubiquitination state facilitates rAAV transduction. Similar to the polarized human airway cells, rAAV transduction in human primary confluent fibroblasts was also augmented by tripeptide proteasome inhibitors. 80% confluent human primary fibroblasts were infected with AV.GFP3ori at an moi of

1000 DNA particles/cell. Figure 16A depicts GFP transgene expression in the absence (left photographs) and presence of 40 μM LLnL (right photographs) at 96 hours post-infection. Similar effects were achieved with 4 μM Z-LLL (data not shown). Top and bottom panels represent bright field and FITC-channel fluorescent photomicrographs, respectively. The mean (+/-SEM, N=3) percentage of cells transduced with rAAV, as measured by FACS sorting of GFP expressing cells, is presented in the bar graph of Figure 16B. Figure 16C demonstrates the identification of the ubiquitinated AAV capsid proteins (marked by arrowhead) 6 hours after infection of primary confluent fibroblasts. In this study, rAAV from infected cells was first immunoprecipitated with anti-VP1,2,3 (AAV-2 capsid) monoclonal antibody followed by Western blot detection of ubiquitin side chains using an anti-ubiquitin monoclonal antibody. The two major background bands migrating at approximately 65 and 25 kd represent heavy and light chain antibody subunits which cross-react with secondary antibodies. Additionally, the equal intensity of lower molecular weight cross-reactive bands (30-40 kd) serve as internal controls for equal loading of protein. Figure 16D demonstrates augmentation of rAAV transduction in polarized airway epithelia by inhibitors of ubiquitin E3 ligase. Epithelia were infected with AV.GFP3ori (10,000 particles/cell) from the basolateral surface following treatment with ubiquitin ligase inhibitor dipeptides (0.2 mM H-Leu-Ala-OH and 0.2 mM H-His-Ala-OH). Results demonstrate the mean (+/- SEM, N=3) number of GFP expressing cells per 10x field at 1 and 15 days post-infection.

C6 cont'd

Please replace the paragraph beginning at page 18, line 15 with the following paragraph:

C7

Figures 17A-F. Persistent induction of rAAV mediated gene transfer in mouse conducting airways by proteasome inhibitors. Figures 17A-C) Recombinant AV.Alkphos (5×10^{10} particles) was administered to mouse lung either as virus alone in PBS or virus in combination with 40 μM LLnL in PBS. Virus was directly instilled into C57Balb/c mice trachea with a 30 G needle in a total volume of 30 μl. To insure the spread of the virus in mouse lung, 50 μl air was pumped into lung through the same syringe immediately after virus was administered. Ninety days after infection, lungs were

harvested intact and fixed in 4% paraformaldehyde followed by cryosection. AAV-mediated transgene expression was evaluated by 10 μm tissue sections staining for heat-resistant alkaline phosphatase. Figure 17A: infection with AAV alone; Figures 17B and 17C: infection with AAV supplemented with 40 M of LLnL. Figures 17D-F) 6 week old BALB/c mouse (N=3 animals in each group) were infected with 5 x 10^{10} DNA particles of AV.LacZ in the absence or presence of 400 μM Z-LLL by nasal instillation. Representative examples of histochemical staining for LacZ expression in large bronchioles 150 days post-infection are shown in Figures 17D and 17E. The right and left sides of each panel represent Nomarski and bright field photomicrographs, respectively. The 100 μm scale bar applies to all photomicrographs. The mean (+/-SEM) percentage of LacZ expressing epithelial cells at various levels of the airway was quantitated using the morphometric procedures outlined in the methods, and the analysis represents results from three independent animals for each group (Figure 17F).

C7 cont'd

Please replace the paragraph beginning at page 19, line 4 with the following paragraph:

C8

Figures 18A-D. Cy3-labeled rAAV infection in Hela cells. Hela cells were infected with Cy3-labeled AV.GFP3ori at an MOI of 500 particles/cells for 90 minutes at 4°C in the absence of serum. Cells were then washed and either directly fixed in 2% paraformaldehyde for 10 minutes or incubated at 37°C for an additional 60 or 120 minutes prior to fixation. Prior to viral infections, cells were incubated for 30 minutes in 1 μM 5-chloromethylfluorescein diacetate (Cell Tracker™ Green CMFDA, Molecular Probe) to allow for visualization of cells and labeled virus in dual fluorescent channel images. Representative confocal image of cells infected for 90 minutes at 4°C followed by a 60 and 120 minute incubation at 37°C are shown for both Cy3 and dual Cy3/FITC channels (Figure 18A). The nuclei in dual channel images are marked by Nu. The confocal images shown were merged from three 0.5 μm layers taken within the central region of the cell. Figure 18B depicts non-confocal images with Nomarski and Cy3 channels for cells infected for 90 minutes at 4°C (left side of Figure 18B) followed by a 120 minute incubation at 37°C (right side of Figure 18B). Virus binding at 4°C localizes

to the surface membrane of the cell. With increased incubation time at 37°C, virus was translocated to the nuclear membrane. Viral binding at 4°C was also competed by the addition of free heparin at the indicated concentrations shown in Figure 18C (confocal images shown from three merged layers). Endocytosis of FITC-labeled transferrin and Cy3-labeled rAAV was observed in Hela cells (Figure 18D). Hela cells were infected with Cy3 rAAV in the presence of FITC-labeled transferrin for 90 minutes at 4°C followed by washing. Cells were then placed at 37°C for 30 minutes prior to fixation and analysis by confocal microscopy. Images in Figure 18D Panel D} were a single 0.5 μm cross section for Cy3, FITC, and combined channels (merged). Results demonstrate colocalization of rAAV and transferrin in the majority of endocytic vesicles.

C8 cancel